

This Page Is Inserted by IFW Operations  
and is not a part of the Official Record

## **BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning documents *will not* correct images,  
please do not report the images to the  
Image Problem Mailbox.**

- 3 -

Gmachl *et al.*  
Appl. No. 09/893,443*Amendments*

This listing of claims will replace all prior versions, and listings of claims in the application.

1-27. (cancelled)

28. (currently amended) A method for identifying a compound that inhibits the ubiquitination reaction mediated by the Anaphase Promoting Complex (APC), comprising

(a) reacting APC11, ubiquitin, a ubiquitin activating enzyme (E1), a ubiquitin conjugating enzyme (E2), ATP, and a test compound under conditions and duration of time sufficient to obtain a measurable level of multiubiquitin chains in the absence of said test compound; and

(b) ~~determining said test compound's ability to interfere with the formation of multiubiquitin chains by APC11~~ comparing the level of multiubiquitin chains formed in the presence of said test compound to the level of multiubiquitin chains formed in the absence of said test compound;

wherein said reaction is made in the absence of APC subunits other than APC11.

29. (cancelled)

30. (currently amended) The method of claim 29 28, wherein said APC11 is human.

31. (currently amended) The method of claim ~~29~~ 28, wherein said E1 is wheat UBA1.

32. (currently amended) The method of claim 29 28, wherein said E2 is the human variant UBCH5b.

- 4 -

Gmachl *et al.*  
Appl. No. 09/893,443

33. (currently amended) The method of claim 29 28, wherein the formation of multiubiquitin chains is measured using an antibody.
34. (previously presented) The method of claim 33, wherein said antibody is specific for APC11.
35. (previously presented) The method of claim 33, wherein said antibody is specific for ubiquitin.
36. (previously presented) The method of claim 33, wherein said antibody is labeled for detection.
37. (previously presented) The method of claim 36, wherein said antibody is labeled with a fluorimetric label, a radioactive label, or an enzymatic label.
38. (currently amended) The method of claim 29 28, wherein said formation of multiubiquitin chains is measured using fluorescence resonance energy transfer (FRET).
39. (currently amended) The method of claim 29 28, wherein one or more assay component selected from the group consisting of APC11, E1, E2 and ubiquitin is fused to an affinity tag.
40. (previously presented) The method of claim 39, wherein said affinity tag is glutathione S-transferase, maltose binding protein, His(6), myc-epitope, biotin, or HA-epitope.
41. (previously presented) The method of claim 39, wherein said assay component fused to said affinity tag is APC11.
42. (previously presented) The method of claim 39, wherein said assay component fused to said affinity tag is E2.
43. (previously presented) The method of claim 39, wherein said assay component fused to said affinity tag is ubiquitin.

- 5 -

Gmachl *et al.*  
Appl. No. 09/893,443

44. (previously presented) The method of claim 39, wherein more than one assay component is fused to said affinity tag.
45. (previously presented) The method of claim 39, wherein said assay component is detected with an antibody specific for said affinity tag.
46. (currently amended) A method for identifying a compound that inhibits the ubiquitination reaction mediated by the Anaphase Promoting Complex (APC), comprising
- (a) reacting APC11, ubiquitin, an APC substrate, a ubiquitin activating enzyme (E1), a ubiquitin conjugating enzyme (E2), ATP, and a test compound under conditions and duration of time sufficient to obtain a measurable level of multiubiquitin chains on said substrate in the absence of said test compound; and
  - (b) ~~determining said test compound's ability to interfere with the formation of multiubiquitin chains on said substrate by APC11~~ comparing the level of multiubiquitin chains formed in the presence of said test compound to the level of multiubiquitin chains formed in the absence of said test compound;
- wherein said reaction is made in the absence of APC subunits other than APC11.
47. (cancelled)
48. (currently amended) The method of claim 47 46, wherein said APC11 is human.
49. (currently amended) The method of claim 47 46, wherein said APC substrate is CyclinB.
50. (currently amended) The method of claim 47 46, wherein said APC substrate is Securin.
51. (currently amended) The method of claim 47 46, wherein said E1 is wheat UBA1.

- 6 -

Gmachl *et al.*  
Appl. No. 09/893,443

52. (currently amended) The method of claim 47 46, wherein said E2 is the human variant UBCH5b.

53. (currently amended) The method of claim 47 46, wherein the formation of multiubiquitin chains is measured using an antibody.

54. (previously presented) The method of claim 53, wherein said antibody is specific for APC11.

55. (previously presented) The method of claim 53, wherein said antibody is specific for ubiquitin.

56. (previously presented) The method of claim 53, wherein said antibody is labeled for detection.

57. (previously presented) The method of claim 56, wherein said antibody is labeled with a fluorimetric label, a radioactive label, or an enzymatic label.

58. (currently amended) The method of claim 47 46, wherein said formation of multiubiquitin chains is measured using fluorescence resonance energy transfer (FRET).

59. (currently amended) The method of claim 47 46, wherein one or more assay component selected from the group consisting of APC11, E1, E2 and ubiquitin is a fused to an affinity tag.

60. (previously presented) The method of claim 59, wherein said affinity tag is glutathione S-transferase, maltose binding protein, His(6), myc-epitope, biotin, or HA-epitope.

61. (previously presented) The method of claim 59, wherein said assay component fused to said affinity tag is APC11.

62. (previously presented) The method of claim 59, wherein said assay component fused to said affinity tag is E2.

- 7 -

Gmachl *et al.*  
Appl. No. 09/893,443

63. (previously presented) The method of claim 59, wherein said assay component fused to said affinity tag is ubiquitin.

64. (previously presented) The method of claim 59, wherein more than one assay component is fused to said affinity tag.

65. (previously presented) The method of claim 59, wherein said assay component is detected with an antibody specific for said affinity tag.

66. (currently amended) A method for identifying a compound that inhibits the self-ubiquitination reaction mediated by the Anaphase Promoting Complex (APC), comprising

(a) reacting APC11, ubiquitin, a ubiquitin activating enzyme (E1), a ubiquitin conjugating enzyme (E2), ATP, and a test compound under conditions and duration of time sufficient to obtain a measurable level of ubiquitination of APC11 in the absence of said test compound; and

(b) ~~determining said test compound's ability to interfere with the ubiquitination of APC11~~ comparing the level of ubiquitination of APC11 in the presence of said test compound to the level of ubiquitination of APC11 in the absence of said test compound.

67. (cancelled)

68. (currently amended) The method of claim 67 66, wherein said APC11 is human.

69. (currently amended) The method of claim 67 66, wherein said E1 is wheat UBA1.

70. (currently amended) The method of claim 67 66, wherein said E2 is the human variant UBCH5b.

71. (currently amended) The method of claim 67 66, wherein said ubiquitination of APC11 is measured using an antibody.

- 8 -

Gmachl *et al.*  
Appl. No. 09/893,443

72. (previously presented) The method of claim 71, wherein said antibody is specific for APC11.
73. (previously presented) The method of claim 71, wherein said antibody is specific for ubiquitin.
74. (previously presented) The method of claim 71, wherein said antibody is labeled for detection.
75. (previously presented) The method of claim 74, wherein said antibody is labeled with a fluorimetric label, a radioactive label, or an enzymatic label.
76. (currently amended) The method of claim ~~67~~ 66, wherein said formation of multiubiquitin chains is measured using fluorescence resonance energy transfer (FRET).
77. (currently amended) The method of claim ~~67~~ 66, wherein one or more assay component selected from the group consisting of APC11, E1, E2 and ubiquitin is a fused to an affinity tag.
78. (previously presented) The method of claim 77, wherein said affinity tag is glutathione S-transferase, maltose binding protein, His(6), myc-epitope, biotin, or HA-epitope.
79. (previously presented) The method of claim 77, wherein said assay component fused to said affinity tag is APC11.
80. (previously presented) The method of claim 77, wherein said assay component fused to said affinity tag is E2.
81. (previously presented) The method of claim 77, wherein said assay component fused to said affinity tag is ubiquitin.

- 9 -

Gmachl *et al.*  
Appl. No. 09/893,443

82. (previously presented) The method of claim 77, wherein more than one assay component is fused to said affinity tag.

83. (previously presented) The method of claim 77, wherein said assay component is detected with an antibody specific for said affinity tag.